Cloning, expression, purification, and biophysical characterization of SSB from pathogenic bacteria

Week 6 – Testing SSB DNA binding
EMSA- Electrophoretic Mobility Shift Assay

Objective: To test whether the SSB protein purified in the previous class can bind to single stranded DNA (ssDNA).

Step 1. Make a 10% Polyacrylamide Gel with either TAE or TBE buffer.

10% PAGE (10 mL total):

DD water	– 6.4 mL
40% acrylamide	– 2.5 mL
10X TAE or TBE	– 1.0 mL
10% APS	– 100 uL
*TEMED	– 10 uL

(mix everything and add Temed last –right before pouring the gel)

Make sure to wash and clean your plates and combs before pouring the gel. Pour the gel, immediately place the comb and allow the gel to polymerize for 15 minutes.

Step 2. Measure the concentration of the SSB protein.
Perform a wavelength scan (240 – 350 nm) in the spectrophotometer (spec) using the SSB storage buffer as blank.

Make a 1:10 dilution of your protein stock (100 ul of protein + 900 ul of storage buffer) in the cuvette. Use a parafilm cover and gently invert a few times to mix the solution.

Place the cuvette in the spec and perform the sample scan. Note down the reading at 280 nm.

Calculate the protein concentration: Usig Beer's law, $Abs=\varepsilon Cl$ $M^{-1}cm^{-1}$

(Abs/ε)*dilution factor = [SSB] M

ε Use the extinction coefficient value you have calculated for the SSB tetramer

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[DNA] = 100 nM
[SSB] = 0 – 1 uM
Buffer = 20 mM Tris-Cl, pH 8.3, 200 mM NaCl
Loading Buffer = 20 mM Tris-Cl, pH 8.3, 200 mM NaCl and 50 % v/v Glycerol

Follow the attached table to mix the various samples.

Let the samples incubate at room temperature for 10 minutes.

Pipette 20 ul of the sample into a new Eppendorf tube and add 20 ul of the loading buffer.

Load 30 ul the sample onto the gel.

Run the gel at 100 Volts in 1X TAE or 1X TBE.

Wait for the colored DNA to get down about 3/4ths of the distance.

Stop the gel and take the gel up to the STORM imager and analyze the data.

Quantitate the bound versus unbound fractions.

(Bound/(bound+unbound)) * [DNA] = amount of DNA bound by the SSB protein.

Plot amount of DNA bound versus [SSB] and obtain the K_D for the interaction.